Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Multiple myeloma is a disease in which conventional chemotherapy has only limited value, but which may be ideal for treatment with passive antibody against a suitable cell surface antigen on the neoplastic plasma cell. The **CD38** antigen is known to be present on the majority of neoplastic plasma cells, and this was confirmed by detailed examination of bone marrow aspirates from three patients. Strong expression of **CD38** was confined to cells which, by the criteria of light-scattering profiles and possession of cytoplasmic Ig, were plasma cells. The vast majority of neoplastic plasma cells appeared to be involved. Using a cell line as a model, it was found that the **CD38** antigen acts as a target for a **chimeric** antibody prepared from the antibody OKT10. The **chimeric** antibody consists of the Fab portion of the mouse monoclonal antibody linked by a stable thioether bond to an Fc molecule derived from human IgG1, thereby forming mouse Fab-human Fc. In contrast to the parent antibody, the **chimeric** molecule mediates antibody-dependent cellular **cytotoxicity** (ADCC) very efficiently with human blood mononuclear effector cells, and is effective at low concentration. Also, even though the **CD38** antigen is present on natural killer cells, there appears to be little deleterious action of the antibody on effector cell function. The antibody also failed to affect the growth of progenitor cells of the granulocyte/macrophage or erythroid lineages present in normal bone marrows, despite the suspicion that these cells express the antigen. Other advantages of the **CD38** molecule are that it is not found in the serum of patients with myeloma, and it does not appear to modulate in vitro. Fourteen patients with florid myeloma and on various chemotherapeutic regimes had an undiminished capacity to mediate ADCC with the **chimeric** antibody, when compared with normal individuals. The maintenance of ADCC activity, coupled with the known suppression of the antibody response in these patients, augers well for treatment with **chimeric** antibody.

Superantigen-directed lysis of multidrug resistant leukemic cell lines (M eting abstract).

Zehrer; Ihle; Beck; Holzer; Orlikowsky; Dohlsten; Kalland; Niethammer; Dannecker

Children's Univ. Hosp., 72076 Tubingen, Germany
Proc Annu Meet Am Assoc Cancer Res 1996, 37, ISSN 0197-016X
Document Type: MEETING ABSTRACTS
Languages: ENGLISH
Main Citation Owner: NOTNLM
Record type: Completed

Superantigen-activated T cells can be targeted by monoclonal antibodies to lyse MHC class II tumor cells (superantigen-dependent cellular **cytotoxicity**, SDCC). We examined the susceptibility of the T-lymphoblastoid leukemic cell line CCRF-CEM and the multidrug resistant (MDR)1-gene product overexpressing sublines CCRF-VCR100, CCRF-VCR1000 and CCRF-ADR5000 to SDCC. A recombinant **fusion** protein of protein A and the superantigen Staphylococcus enterotoxin A (SEA) was used together with the monoclonal antibodies anti-CD7, anti- **CD38**, anti-CD45 and 4E3 (anti-P-glycoprotein) to correlate susceptibility to SDCC with expression of the MDR1-gene product. Our results demonstrate that susceptibility to SDCC is independent of MDR1-gene expression, but is correlated with expression of cell surface antigens on target cells. SDCC could therefore be an alternative immunotherapeutic approach in patients suffering from multidrug resistant relapses of leukemic disease.

Set	Items	Description
S1	96	GENETIC (3N) IMMUNOTOXIN?
S2	59	RD (unique items)
S3	46	S2 NOT PY>1998
S4	182	(HISTONE OR POLYLYSINE) (S) (FUSION OR FUSE? OR CHIMERIC) - (S) (ANTIBOD? OR SCFV)
S5	104	S4 NOT PY>1997
S6	120	S4 NOT PY>1998
S7	16	S6 NOT S5
S8	4	RD (unique items)
S9	27	S5 NOT PY<1994

S10	11	RD (unique items)
S11	0	CD38 (5N) (FUSION OR FUSED OR CHIMERIC) (5N) (TOXIN? OR IMMUNOTOXIN? OR RICIN OR EXOTOXIN? OR ENDOTOXIN?)
S12	666	CD38 (S) (TOXIN? OR RICIN OR EXOTOXIN? OR ENDOTOXIN? OR CYTOTOXIC?)
S13	42	S12 (S) (CHIMERIC? OR FUSED OR FUS? OR FUSION)
S14	14	RD (unique items)
S15	10	S14 NOT PY>1998

Design of a genetic immunotoxin to eliminate toxin immunogenicity.

Chen; Zani; Khouri; Marasco

Dept. of Pathology, Div. of Human Retrovirology, Dana-Farber Cancer Inst., 44 Binney Street, Boston, MA 02115

Gene Ther 1995, 2 (2) p116-23, ISSN 0969-7128

Document Type: JOURNAL ARTICLE

Languages: ENGLISH

Main Citation Owner: NOTNLM

Record type: Completed

Host **antibody** response to toxin molecules is a major obstacle to the use of immunotoxins as efficacious agents in the treatment of human cancer and other diseases. In this study, a genetic form of immunotoxin has been designed which should eliminate toxin immunogenicity by replacing the toxin protein moiety with weakly immunogenic or nonimmunogenic plasmid DNA. A recombinant bifunctional **fusion** protein, which consists of a human **antibody** Fab targeting moiety (directed against gp120, the envelope glycoprotein of human immunodeficiency virus [HIV-1]) and a human DNA binding moiety (**protamine**), is used as a gene carrier. Toxin plasmid DNAs expressing the catalytic fragment of Pseudomonas exotoxin A (PEA) statically interact with the **fusion** proteins to form soluble protein-DNA complexes. The complexes are specifically transferred into HIV-1-infected cells by receptor-mediated endocytosis, resulting in selective killing of the target cells. These 'genetic immunotoxins' may have significant advantages over protein immunotoxins for the treatment of a variety of human diseases. (35 Refs)

Design of a genetic immunotoxin to eliminate toxin immunogenicity.

Chen S Y; Zani C; Khouri Y; Marasco W A

Department of Pathology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

Gene therapy (ENGLAND) Mar 1995, 2 (2) p116-23, ISSN 0969-7128 Journal Code: 9421525

Contract/Grant No.: AI31783; AI; NIAID; AI33802; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Host **antibody** response to toxin molecules is a major obstacle to the use of immunotoxins as efficacious agents in the treatment of human cancer and other diseases. In this study, a genetic form of immunotoxin has been designed which should eliminate toxin immunogenicity by replacing the toxin protein moiety with weakly immunogenic or nonimmunogenic plasmid DNA. A recombinant bifunctional **fusion** protein, which consists of a human **antibody** Fab targeting moiety [directed against gp120, the envelope glycoprotein of human immunodeficiency virus (HIV)-1] and a human DNA binding moiety (**protamine**), is used as a gene carrier. Toxin plasmid DNAs expressing the catalytic fragment of Pseudomonas exotoxin A (PEA) statically interact with the **fusion** proteins to form soluble protein-DNA complexes. The complexes are specifically transferred into HIV-1-infected cells by receptor-mediated endocytosis, resulting in selective killing of the target cells. These 'genetic immunotoxins' may have significant advantages over protein immunotoxins for the treatment of a variety of human diseases.

Single-chain antibody-mediated gene delivery into ErbB2-positive human breast cancer cells.

Li X; Stuckert P; Bosch I; Marks J D; Marasco W A

Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, Massachusetts 02115, USA.

Cancer gene therapy (England) Aug 2001, 8 (8) p555-65, ISSN 0929-1903 Journal Code: 9432230

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Too late

Targeted gene transfer by nonviral vectors can be achieved through incorporation of specific ligand(s) into the vectors. In this study, the effects of incorporation of an anti-ErbB2 single-chain **antibody** fragment (**ScFv**) into nonviral vectors for targeted gene delivery were investigated. The ML39 **ScFv** , selected from a human **ScFv** phage display library and affinity matured in vitro (K(d)=1 x 10(-9) M), was used as ligand specific for the extracellular domain of the tumor surface protein, ErbB2. Two approaches were taken: (a) development of a vector that is composed of a bifunctional **fusion** protein capable of binding DNA with the ErbB2-specific ML39 **ScFv** at its N-terminus and a truncated form of human **protamine** at its C-terminus, and (b) formulation and evaluation of delivery vectors consisting of three independent components including ML39 **ScFv** , **protamine** , and cationic lipids. We demonstrate that **fusion** proteins comprised of the ML39 **ScFv** and a truncated form of **protamine** , denoted as **ScFv** -P-S, can selectively deliver exogenous DNA into ErbB2(+) cells, with an 8- to 10-fold increase in expression levels of the luciferase reporter gene in ErbB2(+) cells as compared to ErbB2(-) cells. In addition, vectors formulated by appropriately mixing DNA, **ScFv** , **protamine** , and lipids in vitro could even more efficiently deliver the reporter gene into ErbB2(+) cells with approximately 5-fold increase in gene expression in ErbB2(+) cell as compared to ErbB2(-) cells. Expression and refolding of the **ScFv** **fusion** proteins, in addition to determination of optimal conditions for vector development using these approaches, are discussed.

Novel genetic immunotoxins and intracellular antibodies for cancer therapy.

Chen S Y; Marasco W A

Department of Cancer Biology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina, USA.

Seminars in oncology (UNITED STATES) Feb 1996, 23 (1) p148-53, ISSN 0093-7754 Journal Code: 0420432

Document type: Journal Article; Review; Review, Academic

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In our recent studies we have developed two novel approaches with potential application for cancer therapy. One approach, termed **genetic immunotoxins**, is selectively targeted to the molecules on the cell surface to kill malignant cells. The **genetic immunotoxin** consists of an antibody-DNA-binding protein linked to a toxin expression plasmid DNA, and the selective cell killing of the **genetic immunotoxin** is accomplished by transferring toxin expression DNAs into a target cell. The **genetic immunotoxin** with decreased immunogenicity and increased cytotoxicity may have significant advantages over currently described recombinant protein immunotoxins for cancer therapy. Another approach, termed intracellular antibodies, is targeted to inactivate an oncoprotein molecule inside cells to block the malignant growth by intracellular expression of engineered antibodies. We briefly illustrate the design of the two approaches and their potential for clinical applications.

Targeting DNA through fibroblast growth factor receptors (Meeting abstract).

Sosnowski; Gonzalez; Buechler; Amburn; Klausen; Wang; Chandler; Baird

PRIZM Pharmaceuticals, San Diego, CA 92121

Proc Annu Meet Am Assoc Cancer Res 1996, 37, ISSN 0197-016X

Document Type: MEETING ABSTRACTS

Languages: ENGLISH

Main Citation Owner: NOTNLM

Record type: Completed

A mitotoxin consisting of basic fibroblast growth factor (FGF2) and saporin (SAP), a ribosome-inactivating protein (RIP), can eliminate cells overexpressing FGF receptors. To increase specificity, we are exploring the possibility of using FGF2 to deliver DNA into cells. Targeting plasmid DNA encoding SAP provides a means of introducing transcriptional control over its RIP activity. Delivery of genes through the FGF receptor was first validated using DNA encoding beta-galactosidase (beta-gal). FGF2 was chemically conjugated to **polylysine** (pLys) and then condensed with the plasmid pSV-beta. FGF2-pLys conjugates were equipotent to FGF2 in stimulating endothelial cell proliferation. FGF-pLys-DNA complex formation and beta-gal expression in target cells were evaluated by gel-shift assays to monitor FGF-pLys binding to DNA, toroid formation to monitor DNA condensation, and cell staining and enzyme assays to monitor beta-gal expression. beta-gal expression in COS cells increased in a time-and dose-dependent manner and was highest with complexes containing lysine polymers of at least 84 residues, protein/DNA mass ratios of 2:1, and DNA concentrations of 540 pM. Treatment with chloroquine increased expression 8-fold and inclusion of endosome disruptive peptides increased expression 26-fold. Specificity of delivery through the FGF receptor was demonstrated by competition with FGF2, heparin, and FGF2 neutralizing **antibodies**. Targeting of beta-gal DNA was also demonstrated in BHK, NIH-3T3 and B16 cells, all of which have FGF2 receptors. Therefore DNA can be introduced into cells via FGF receptors, suggesting that receptor-mediated delivery of DNA encoding RIPs could provide a more specific alternative to **fusion** protein mitotoxins.

Engineered anti-CD38 monoclonal antibodies for immunotherapy of multiple myeloma.

Ellis J H; Barber K A; Tutt A; Hale C; Lewis A P; Glennie M J; Stevenson G T; Crowe J S

Molecular Immunology Group, Wellcome Foundation Ltd, Beckenham, Kent, United Kingdom.

Journal of immunology (Baltimore, Md. : 1950) (UNITED STATES) Jul 15 1995, 155 (2) p925-37, ISSN 0022-1767 Journal Code: 2985117R

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Multiple myeloma is a malignancy of plasma cells for which there is no effective treatment. To develop an immunotherapeutic agent, we have raised a high affinity mAb (AT13/5) against **CD38** , one of the few well-characterized surface Ags present on myeloma cells. Since murine monoclonals have many disadvantages as human therapeutics, we prepared two engineered forms of the Ab: a CDR-grafted humanized IgG1 and a **chimeric** FabFc2 (mouse Fab cross-linked to two human gamma 1 Fc). To retain affinity in the humanized Ab, a number of changes were required to the human framework regions of the heavy chain. In particular, through systematic mutagenesis and computer modeling, we identified a critical interaction between the side chains of residues 29 and 78, which may be important for the humanization of other Abs. The properties of the humanized IgG1 and FabFc2 constructs were compared in a series of in vitro tests. Both constructs efficiently directed Ab-dependent cellular **cytotoxicity** against **CD38** -positive cell lines, but C was activated only poorly. Neither construct caused down-modulation of **CD38** , nor did they affect the NADase activity of **CD38** . Despite their differing structures, both Abs showed similar activity in most assays, although the humanized IgG1 was more potent at inducing monocyte **cytotoxicity** . These data represent the first direct comparison of CDR-grafted and **chimeric** FabFc2 forms of the same Ab, and offer no support for the perceived advantages of the FabFc2. These Abs show promise for therapy of multiple myeloma and other diseases involving **CD38** -positive cells.

Antibody-targeted superantigens induce lysis of major histocompatibility complex class II-negative T-cell leukemia lines.

Ihle J; Holzer U; Krull F; Dohlsten M; Kalland T; Niethammer D; Dannecker G E

Department of Oncology/Hematology, Children's University Hospital, Tubingen, Germany.

Cancer research (UNITED STATES) Feb 1 1995, 55 (3) p623-8, ISSN 0008-5472 Journal Code: 2984705R

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

CTLs bearing certain T-cell receptor V beta-regions are directed by the bacterial superantigen Staphylococcus enterotoxin A (SEA) to lyse MHC class II-positive cells. In order to extend superantigen-dependent **cytotoxicity** to MHC class II-negative carcinoma cells, covalent conjugates of superantigen and mAbs against surface markers of these cells have been used. We now describe a novel strategy which allows rapid selection of mAb suitable for superantigen targeting against MHC class II-negative tumor cells. A recombinant **fusion** protein of protein A and SEA binding to the mAbs CD7 or **CD38** was able to mediate T cell-dependent lysis of MHC class II-negative Molt-4 and CCRF-CEM acute lymphatic leukemia cell lines. Lysis was dose dependent and correlated with E:T cell ratio. In contrast, SEA alone did not induce any significant lysis. In order to decrease the MHC class II affinity of the protein A-SEA complex, a point mutation was introduced into SEA (protein A-SEA mu9). The mutated **fusion** protein had similar potency as protein A-SEA against Molt-4 cells but was 100-fold less active against MHC class II-positive cells. Considering the efficiency and specificity of the mutated SEA protein interacting with mAb in targeting T lymphocytes against MHC class II-negative leukemia cells while only marginally affecting normal MHC class II-positive cells, we suggest the development of SEA-mAb **fusion** proteins as a potential adjuvant therapy of leukemias.

Preliminary studies for an immunotherapeutic approach to the treatment of human myeloma using chimeric anti-CD38 antibody.

Stevenson F K; Bell A J; Cusack R; Hamblin T J; Slade C J; Spellerberg M B; Stevenson G T

Lymphoma Research Unit, Tenovus Research Laboratory, General Hospital, Southampton, UK.

Blood (UNITED STATES) Mar 1 1991, 77 (5) p1071-9, ISSN 0006-4971 Journal Code: 7603509